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PARTICLE SORTING AND LABIAL PALP FUNCTION IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THUNBERG, 1795)

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Controversy still attends the question whether bivalves exercise selectivity over ingested material. Various interpretations have been advanced, mainly based upon comparison of stomach contents to the suspension in the environment, and the direct observation of various particles on the exposed pallial surfaces. The functional interpretation of the exquisitely complex ciliation of the pallial organs, particularly by Atkins (1937a, 1937b, 1937c) is predicated upon the movement of particular cilia and tracts and their affect upon single particles. The suggestion is made here that the cilia of the ctenidium and palpi act primarily upon mucus and only indirectly on individual particles. Further, it is suggested that the overall functioning of the labial palpi has been hitherto misunderstood.

Particle sorting activities fall into two phases: the segregation of inorganic particles from nutritious ones, and possible selectivity between various organic entities (*i.e.*, bacteria and phytoplankters). Fox (1936) found that *Mytilus californianus* exercised a marked ability to sort inorganic from organic particles, though some inorganic material did become ingested. According to Coe (1947), up to one-half of the stomach contents in *Tivela stultorum* may consist of sand grains 200–300 μ diameter, but this need not be interpreted as a lack of selectivity because interstitial and periphytic micro-organisms may be the principal dietary source and sand may be used for grinding food, as is most probably the case in the carnivorous septibranchs. Oysters generally do not ingest quantities of large mineral particles, though Nelson (1923) stated that under turbid conditions much sand was present in the stomach of *C. virginica*. It has been suggested that sorting, if it occurs, is merely based upon particle size. Jorgensen (1960) found that *Mytilus edulis* was unable to retain large molecules of haemoglobin, but did filter particles as small as

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1μ diameter. These findings conflict with those of Tammes and Dral (1955) who noted a decreasing ability in mussels to retain the smaller particles.

This paper examines the various possible mechanisms involved in the sorting activities of the Pacific oyster and brings forward evidence as to the function of the labial palpi in that species.

MATERIALS AND METHODS

Inhalant and exhalant apertures were measured photographically in living subjects, then oysters of various sizes were relaxed in chloral hydrate solution, and the pallial organs dissected and measured. Pumping volumes were monitored at various temperatures using the Galtsoff (1926) constant-level tank with the Moore (1910) rubber apron supplemented by velocity determinations with heated thermistor probes (McCammon, 1965).

The labial palpi were observed in the entire animal by means of an American Optical Pan Endoscope cystoscope inserted through a small channel cut into the shell margin. The speed and direction of mucus flow were monitored by means of colloidal graphite and sand grains upon the excised palpi, and in whole specimens by careful removal of the anterior portion of the shell. Animals in this condition survived several months and appeared to function normally.

A culture of *Chromatium warmingii* Cohn, 1875 purchased from the American Type Culture Collection (No. 14959) was grown in hydrogen sulphide liquid medium with added vitamin and trace elements. *Chromabacterium,* probably *C. amethystinum* Chester, 1897 was isolated from river water and grown on nutritive glucose agar at 25° C for 5 days. For presentation to the oysters, the *Chromatium* medium was centrifuged at low speed for 10 minutes, and the sedimented bacteria resuspended twice with 0.45 μ filtered sea water. Colonies of *Chromabacterium* were removed from the agar surface with a loop and dispersed by brisk agitation in 25‰ sea water. Test oysters were held in shallow trays maintained at 15° C, and supplied with various suspensions of bacteria, either alone, or mixed with the algae *Tetraselma* and *Chlorella.*

RESULTS

The pallial complex may be considered as a simple pump housed in a chamber (inhalant chamber) provided with a restricted inlet (inhalant aperture) and a larger exit (exhalant aperture). The ctenidium functions as a large diaphragm which is also porous to water. Measurements of three representative oysters are given in Table I and the relationship shows schematically in Figure 1. The relative size of the inhalant and exhalant apertures is of great theoretical importance and in *C. gigas* appears to be contrary to the majority of bivalves where the inhalant aperture is substantially larger. In the Pacific oyster the mantle borders are constantly moving so the relationship is not fixed and much of the exhalant region consists of the promyal chamber exit, where water flow may be negligible though the pallial borders are separated.

Noteworthy is the uniformity of ostial size (*ca.* 2900 μ^2), smaller oysters having fewer, rather than smaller, ostia. An individual of 8.2 cm shell length possessed an average inhalant aperture of 0.51 cm^2 and exhalant aperture of 1.42 cm^2. Total

TABLE I

Measurement of ctenidial apparatus for three Pacific oysters

	A	B	C
Shell length (cm)	3.1	8.2	15.0
Meat weight (g)	6.0	27.2	43.9
Ctenidial length (cm)	1.7	6.0	13.0
Ctenidial width	0.5/0.6	1.2/1.5	1.2/1.7
No. of plicae	580	1300	1760
No. of ostia	3.5×10^5	1.85×10^6	2.69×10^6
Area			
Ctenidium (plain) cm^2	9.6	45.6	79.0
Ctenidium (total)	32.6	119.7	174.0
Ostia μ^2 (each)	2900	2900	2900
Total ostia cm^2	10.1	46.6	78.2
Inhalant aperture mm^2	14	51	85
Exhalant	35	142	243
Width of valve opening cm	0.3	0.4	0.6

potential ostial openings amounted to 46.6 cm^2. It is unlikely that all ostia are fully opened simultaneously but if only 50% are functional a much greater total area for water transportation is presented by the openings of the ctenidium than by the inhalant or exhalant areas.

It is well established that the introduction of a partial obstruction in a fluid flow system results in a pressure rise proximal to the obstruction and a fall on the distal side, so water drawn through the relatively small inhalant passages accelerates, then the flow speed is reduced and the ostial openings are probably negotiated at a very low velocity.

Particles settle under the influence of gravity, behaving according to the Stokes equation, and as particles smaller than 200 μ diameter attain terminal velocity almost instantaneously, the constituents of natural suspensions respond quickly to dynamic changes in their environment. It is assumed that to keep a settling particle in suspension, active agitation, or a constant upward flow component in the medium at least equal to the settlement velocity of the particle in question is needed. The theoretical settlement velocities for various-sized particles of 2.6 specific gravity are shown in Figure 2 together with the maximum pallial velocity for a given pumping rate for an 8-cm oyster. The maximum measured and theoretical inhalant pallial velocity is in the order of 18 mm/min. Describing a trajectory through the inhalant pallial chamber, it is unlikely that any particles of high specific gravity larger than 14 μ diameter would impinge upon the ctenidium, but would fall short to the inner mantle surface. So the chances of heavy inorganic particles reaching the ctenidium are much less than for organic particles which nearly approach the specific gravity of sea water.

It may be concluded that the arrangement of the pallial cavity with a relatively small inhalant aperture and a large total ostial aperture, thereby decreasing approach velocity to the ctenidium, is, in fact, a most efficient settlement chamber.

This gravimetric mechanism may be termed the preliminary sorting method, and observations undertaken upon several other bivalve families and genera show that it is probably universal among the ctenidial Bivalvia. It prevents the impingement of mineral particles upon the delicate tissue of the ctenidium and it is probable that the greater proportion of inorganic material rejected in the pseudofeces has not been in contact with either the ctenidia or labial palpi, but settled directly upon the inhalant chamber mantle surfaces.

Those suspended particles, both organic and inorganic, that successfully cross the low velocity zone of the inhalant pallial chamber, either impinge upon the ctenidium or pass through the ostia and are carried away in the exhalant stream. Once on the ctenidium, particles are entrapped in mucus and transported by the activity of the frontal cilia. There appear to be definite thigmotropic sensors upon the ctenidial filaments, as stimulation of a single filament does not appear to increase the mucus flow, but stimulation of two or more adjacent filaments results in a copious secretion. Ctenidial mucus is elaborated by two distinct types of cells. The outer frontal surfaces of the filaments have many columnar eosinophilic cells, while the lateral regions contain nests of wide goblet cells.

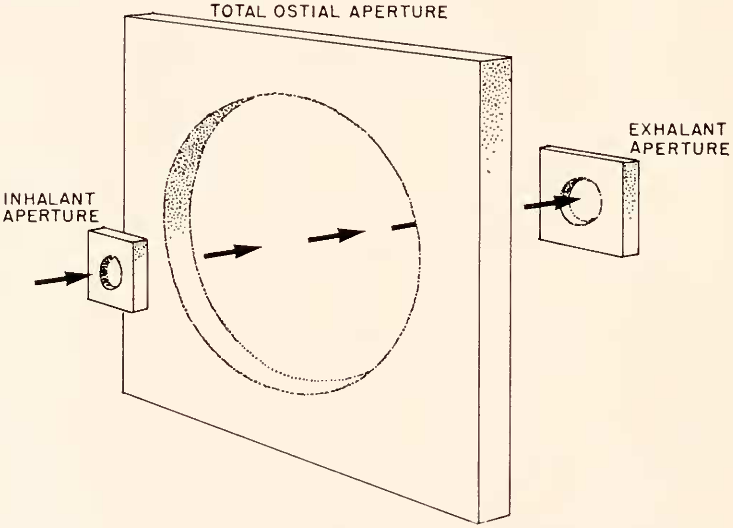

Figure 1. Schematic representation of the relative areas of the inhalant, ostial, and exhalant apertures in the Pacific oyster.

The ctenidial surfaces are normally covered with a watery serous fluid, approximately 5 μ thick, which is not subject to ciliary movements, while a definite band of mucus overlying the frontal cilia forms a zone about 12 μ thick and 20 μ wide on each filament. This is a mucus concerned with particle entrapment and is elaborated by the eosinophilic cells. The ctenidia are extremely sensitive to disturbance, either by tactile, temperature, or light stimulation and react in the abundant production of "rejection" mucus which forms a 250–400 μ sheet over the ctenidium and is elaborated by the laterally situated goblet cells. These mucus masses are invariably carried to the free margins. Mucus entering the terminal grooves to reach the mouth must perform a turn of around 180°. The thick layers of mucus are unable to do this and fall off the ctenidium onto the inner mantle margins where they are rejected as pseudofeces; this separation of unwanted mucus is aided by the activity of the lateral groove cilia.

It is highly probable then that two distinct mucus types are concerned with the entrapment and rejection of particles while gravimetric settlement in the inhalant chamber readily accounts for segregation of organic from inorganic particles, such as the report by Allen (1921) noting separation of a blue-green alga from coarser materials.

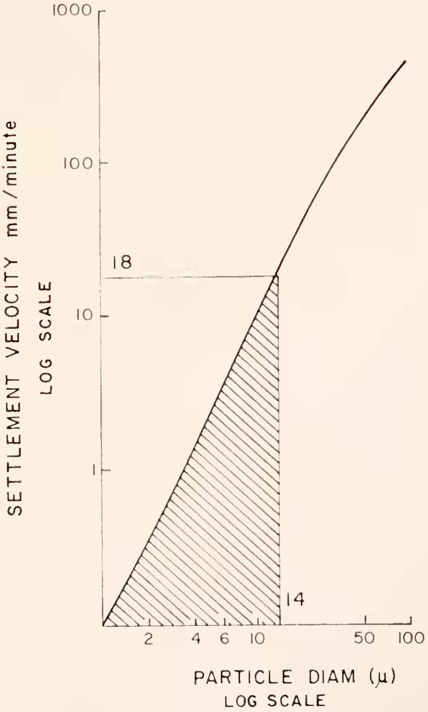

FIGURE 2. Relationship of settling velocity to particle size assuming a specific gravity 2.6 Ordinate 18 is the maximum inhalant pallial velocity for an 8 cm Pacific oyster.

There are a number of reports dealing with separation between various organic forms such as yeasts and algae or algae and bacteria. The central prop to these observations is the report by Loosanoff (1949) that *C. virginica* is able to segregate purple sulphur bacteria from algae. As resolution of this point is central to a better understanding of oyster nutrition, Loosanoff's (1949) experiments were repeated using two genera of chromatophoric bacteria. The autotroph *Chromatium warmingii* is probably identical with the species used by Loosanoff and the other species, a heterotroph, *Chromabacterium cf amethystinum* was also used.

Both bacteria were retained and ingested when presented as pure cultures. If the total load (wet weight) exceeded 200 mg/liter, rejection occurred with production of bright purple pseudofaeces. With lighter loads no rejection was evident and ingestion occurred. If the *Chromatium* was not centrifuged out of the growing medium and washed, substantial to total rejection occurred, probably the result of irritation to the ctenidium by traces of hydrogen sulphide. When mixed with *Tetraselma* ingestion followed, but the bacteria speedily lysolized in the stomach, so that a predominance of algal cells remained. This was more evident with *Chlorella* than *Tetraselma*, the former passing almost undigested through the gut and producing bright green faeces. This clearly demonstrates that the Pacific oyster readily takes up and digests purple sulphur bacteria and only rejects them when their total load exceeds 200 mg/liter.

While ctenidial sorting activities are figured in functional interpretations, the implicated organs have chiefly been the labial palpi. As early as 1851 Alder and Hancock suggested that the palpi of *Pholas* and *Mya* are responsible for active particle sorting. The theory of palp ciliary sorting was much furthered by Kellogg (1915). These studies were made by the observation of the ridged surface and the behavior of small particles placed upon them (Galtsoff 1964), generally with the added insult of brilliant illumination. With this approach, Yonge (1926) found that carmine grains may be transported towards the mouth while carborundum particles are rejected at the edge of the palpi. Menzel (1955) noted similar sorting in *C. virginica* and *O. equestris*. Earlier, Yonge (1923) had put forward the opinion that gravity, causing heavy particles to settle in the interridge grooves,

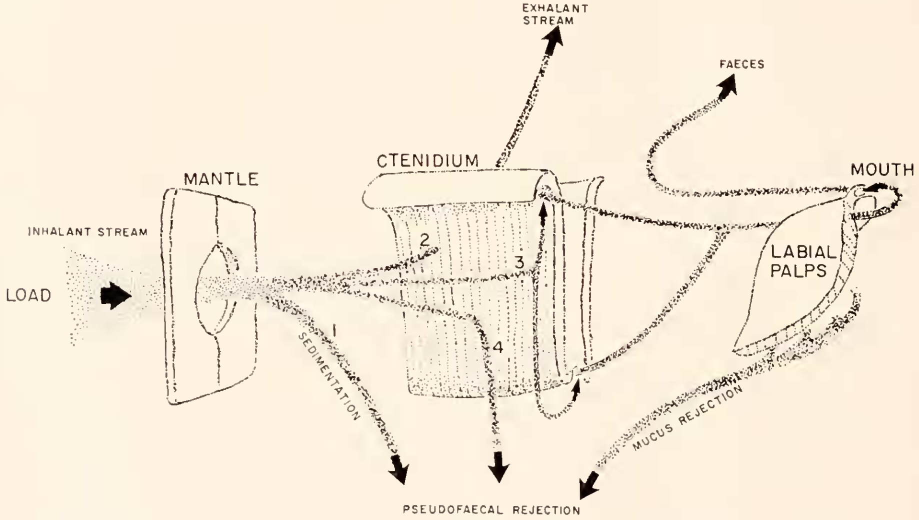

FIGURE 3. Schematic representation of paths and fate of particulate material drawn into the inhalant pallial cavity; (1.) sedimentation of particles of high specific gravity; (2.) passage through the ostium; (3.) impingement upon ctenidium and transportation on frontal mucus bands to food grooves; (4.) rejection of large mucus masses.

resulted in sorting. This is entirely untenable, as at the best only two surfaces can be suitably positioned to take advantage of gravitational separation; furthermore, observations of many different bivalve taxa reveal that in their natural positions the rejecta of the palpi move in an upwards direction.

Observation of the labial palpi by means of a cystoscope shows that in *C. gigas* and other species the two palp lamellae are always closely appressed. When considered as an entity, rather than two separate surfaces, the result of ciliary activity is different. Nelson (1923) found that when narcotized with magnesium sulphate the ridges of the palpi of the oyster collapsed, resulting in large quantities of mucus and food blocking the buccal cavity, and Churchill and Lewis (1924), working on the freshwater *Anodonta,* concluded that the palpi functioned to reduce the quantity of material entering the mouth, but did not discuss the basis for their assertion.

During the course of many hundreds of dissections it was noted that though the food grooves and ctenidia bore light-colored and almost transparent mucus, the mucus of the buccal cavity and stomach was dark brown and contained many more particles.

TABLE II

Dry and wet weights of food groove and buccal mucus from Pacific oysters

	Food groove			Buccal cavity		
	Wet	Dry	% solid	Wet	Dry	% solid
	0.2967	0.0122	4.1	0.1873	0.0237	12.6
	0.3456	0.0177	5.1	0.8380	0.0691	8.2
	0.2536	0.0093	3.6	0.3372	0.0334	9.9
	0.4611	0.0064	1.3	0.5611	0.0592	10.5
	0.3371	0.0082	2.4	0.3825	0.0506	13.2
	0.5200	0.0193	3.7	0.6730	0.1082	16.0
	0.4766	0.0098	2.0	0.4201	0.0566	13.5
$\bar{X}$	0.3843	0.0118	3.1	0.4856	0.0572	12.0

The dissection of actively feeding oysters and rapid removal with a micropipette of samples of the food groove mucus and buccal mucus and their wet and dried weight differential revealed that the ctenidial mucus has an average 3.1% dry weight, but the buccal mucus 12.0%, a fourfold increase in particulate matter. The data for seven oysters are given in Table II.

During water pumping, whether food particles are present or not, a continuous stream of mucus is moving towards the mouth from the ctenidial region. It was established that an individual oyster with an 8.2 cm total shell length has a ctenidial area of approximately 120 cm^2, with 50 cm^2 of this consisting of the mucus bands overlying the frontal cilia. In the undisturbed state at 20° C this mucus travels approximately 131 mm/min. This layer is usually 12 μ in thickness so that it may be calculated that 7.9 cm^3 of mucus would enter the mouth each hour. This is a substantial volume when the stomach capacity is approximately 0.75 cm^3.

DISCUSSION

It is well established that bivalve stomach contents are not representative of the particles suspended in the environment, but there is a degree of selectivity in the particles retained, either a subsequent sorting activity by the pallial organs or the result of non-retention. Other particles, those of greater or lesser specific gravity than sea water, will describe trajectories through the low-velocity zone of the inhalant pallial cavity and are unlikely to impinge upon the ctenidium. Those particles that are retained on the ctenidium are suspended in the frontal mucus bands and carried towards the food grooves. Overstimulation of the filaments by large masses of food or large particles will cause the release of the heavier rejectory mucus which is removed from the ctenidium either by muscular action or by its inability to enter the food grooves. This is the mucus comprising the 'mucus-net' of MacGinitie (1941) and does not represent the normal pumping-feeding configuration. The subjects were either reacting to unfavourable water conditions or microscopic examination was protracted and the ctenidia disturbed by high levels of illumination. Further evidence of their distressed condition is evidenced by the activity of the labial palpi reported by MacGinitie. In the normal feeding situation the palps are appressed, moving only to detach rejection mucus from their free edges.

An explanation of Loosanoff's (1949) observations may lie in the digestive ability of the oyster for bacteria. Both *Chromatium* and *Chromabacterium* placed in filtered stomach juice undergo lysolysis in 6 to 10 minutes. It may be speculated that while both algae and bacteria were being simultaneously ingested, the preferential digestion of bacteria resulted in bacteria-free faeces. The colored pseudofeces show that most of the suspension was being rejected due to the high concentration of H_2S undoubtedly present if *Chromatium* was proliferating. If the bacterial population was sufficiently high the purple colouration would mask the green of the algae, resulting in purple pseudofeces, while the digestive activity of the gut would produce brownish-green faeces. This interpretation is supported by the rapid microscopic examination of gut contents where many bacteria were found.

The literature is almost unanimous in interpreting palp function as sorting unwanted particles prior to ingestion. This is based entirely upon the study of mucus movements over isolated palp surfaces, yet in the normal state the inner surfaces of the palpi are always pressed together resulting in a "sandwich" of mucus between opposing ciliated surfaces. The mucus-bearing food particles are carried completely within the oral groove, so are not affected by the ciliary activity upon the palp ridges. However, the depths of the interfolds bear ciliary tracts that beat away from the central food groove, so there is a trend to remove mucus from the groove axis. If the mucus is free-flowing it will eventually be carried to the palp margin and discarded. If particulate matter is included, the mucus will come under the influence of the other ridge ciliary tracts and be carried to the crests of the folds where it will be transported in an anterior direction parallel to the palp length. A band of cilia on the posterior upper surface of each fold beats in the opposite direction. The net result of this complicated ciliation is to bring any large mucus masses to the free edge of the palp for rejection. Fine mucus is drawn off by the outward-beating cilia at the bottom of the folds and particulate matter is carried over the folds and returned to the food groove.

Ridewood (1903), commenting upon genera with very different ctenidial structure, noted the striking similarity of the labial palpi characterized in most taxa by transverse ciliated ridges borne on two opposed surfaces, the sole exception being in *Solemya*, *Huxleyea*, and the closely related *Nucinella*. In macrophagous forms the palpi are reduced and may be entirely absent, as in the carnivorous septibranch *Cuspidaria*, or reduced to unciliated flaps in *Poromya*. This would indicate the universality of the functioning of these organs in the filter- and deposit-feeding Bivalvia. The particle-concentrating capability of the Pacific oyster palpi is approximately fourfold. Under similar feeding conditions to produce 1 g dry weight material requires 32.6 g of food-groove mucus while only 8.5 g buccal mucus. While the oyster palpi are predominantly mucus reducers they possess the ability to reject their entire load. In a normal functioning state they are covered with a serous mucus similar in consistency to the ctenidial frontal filament mucus, but the subepidermal goblet cells secrete copious quantities of rejectory mucus when stimulated.

Bivalves, encased in their shell envelope, lack external food-gathering organs, with the possible exception of the protobranch palp proboscides and, in some taxa, the foot. They have developed a complex mechanism for bringing food, suspended in the inhalant stream, into the pallial cavity, retaining a portion of these and conveying them to the mouth. The major sorting activity is the segregation and rejection of heavy mineral particles, accomplished due to the architecture of the pallial complex, rather than an intrinsic physical sorting mechanism of the pallial organs. I conclude that ctenidial selectivity is limited to a simple acceptance or rejection of particles and mucus present on a particular lamellar segment, while the major function of the oral palpi is as reducers of mucus volume prior to ingestion. The integrated mechanism proposed for the Pacific oyster is represented schematically in Figure 3.

SUMMARY

1. The pallium of the Pacific oyster comprises a restricted entrance and exit to a chamber separated by the ctenidium, analogous to a large diaphragm pump. This configuration results in a low water velocity in the inhalant cavity.

2. Separation and rejection of particles of high specific gravity occurs prior to impact upon the ctenidium, due to gravitational settlement because of the low pallial water velocity.

3. Particles settling on the ctenidium are either rejected or carried in mucus to the labial palpi where the volume of mucus is reduced, and the concentrated food-mucus mass passed to the mouth.

4. Pacific oysters are not able to sort purple bacteria from unicellular algae, but rapid digestion results in bacteria-free alimentary canal and faeces.

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